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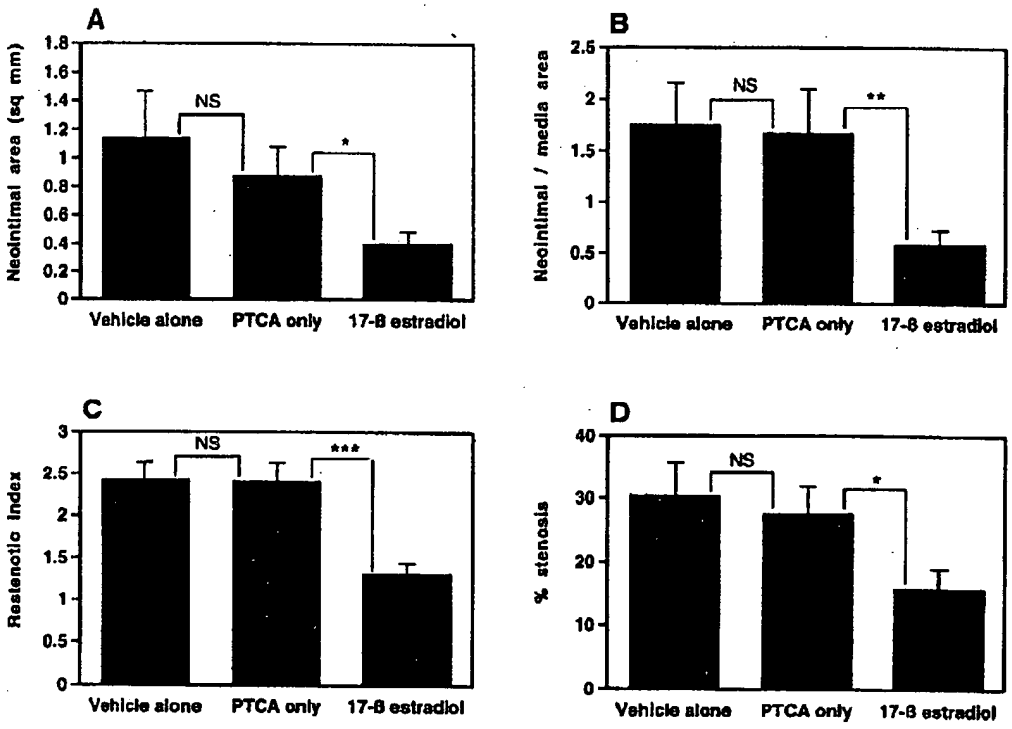
(54) Titre : LA DIFFUSION LOCALE DE 17-BETA ESTRADIOL DIMINUE L'HYPERPLASIE NEOINTIMALE SUITE A UNE ANGIOPLASTIE CORONAIRE SUR UN MODELE PORCIN

(54) Title: LOCAL DELIVERY OF 17-BETA ESTRADIOL DECREASES NEOINTIMAL HYPERPLASIA FOLLOWING CORONARY ANGIOPLASTY IN PORCINE MODEL



(57) Abrégé/Abstract:
Th cardioprotective effects of estrogen are well recognized. In in vitro experiments, and upon systemic administration, 17 - beta estradiol has shown to inhibit vascular smooth muscle cell proliferation and intimal hyperplasia. We hypothesized that locally deliver d 17 - beta estradiol could inhibit neointimal proliferation following balloon angioplasty in porcine coronary arteries. Immunohistochemical, and morphometric analyses revealed that, arterial segments treated with local delivery of 17 - beta estradiol showed significantly less smooth muscle cell proliferation and neointima formation. Compared to PTCA only, or vehicle alon , 17 - beta estradiol decreas d neointima formation by 54.6 % and 64.9 % respectively.

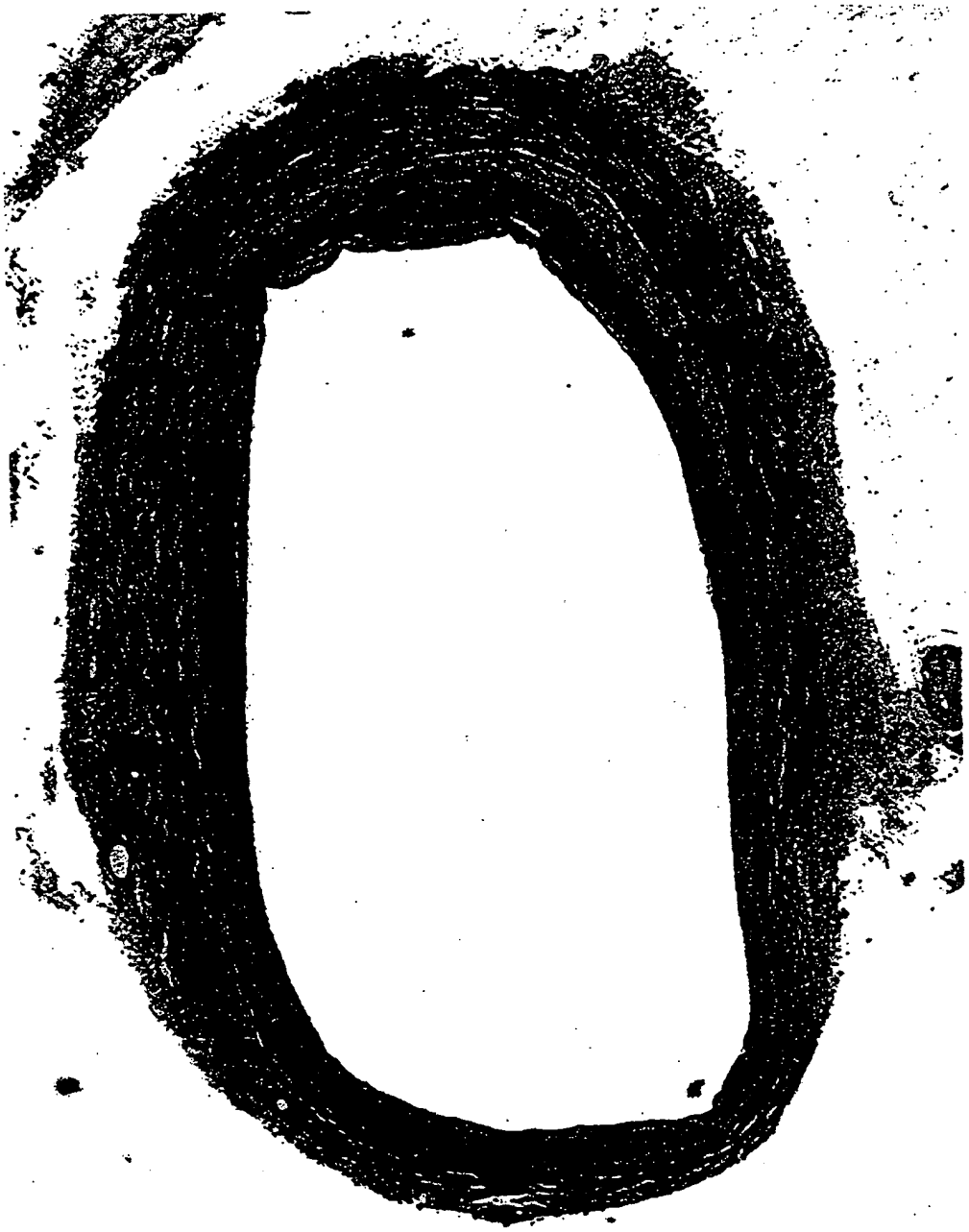


FIGURE 1A

FIGURE 1B

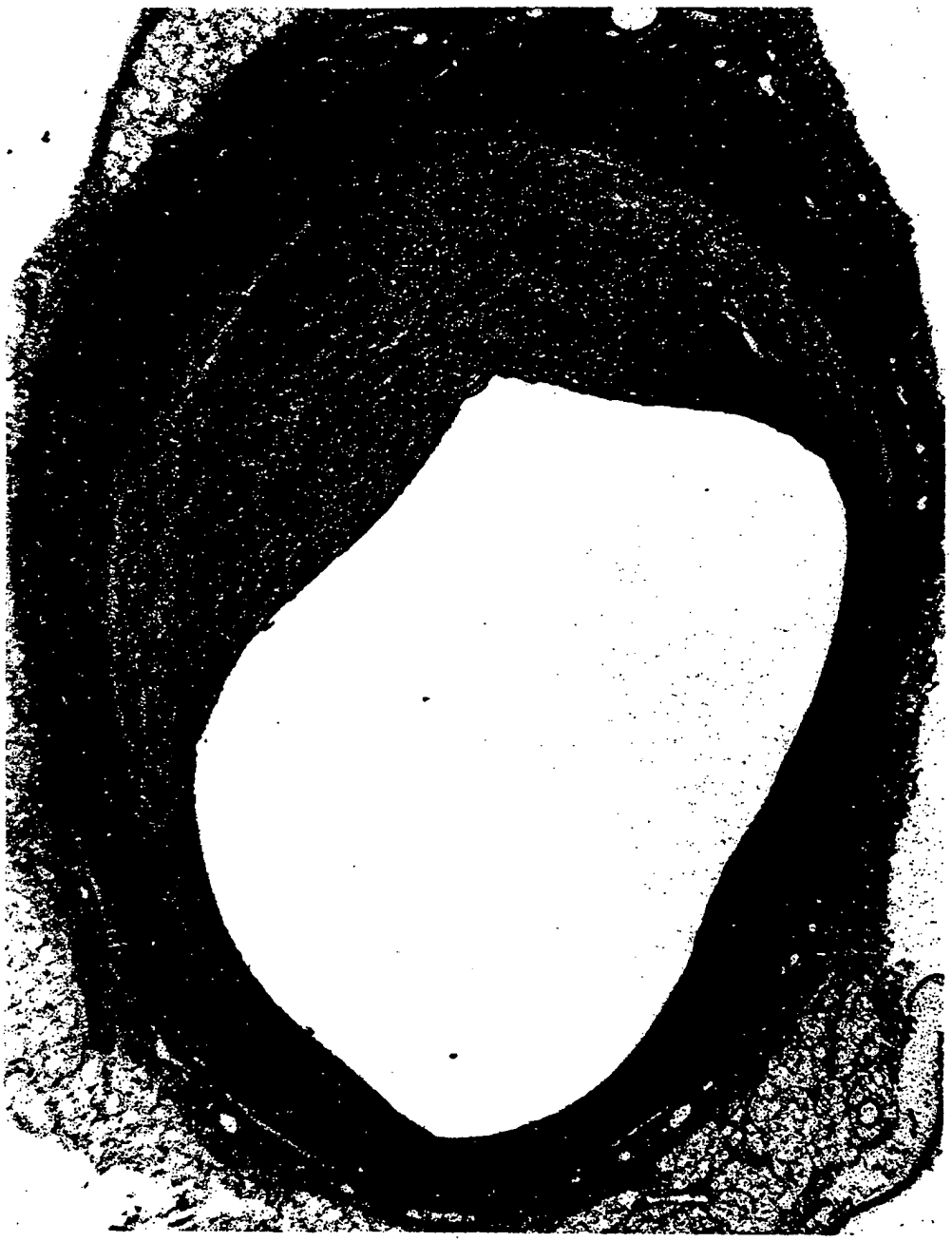
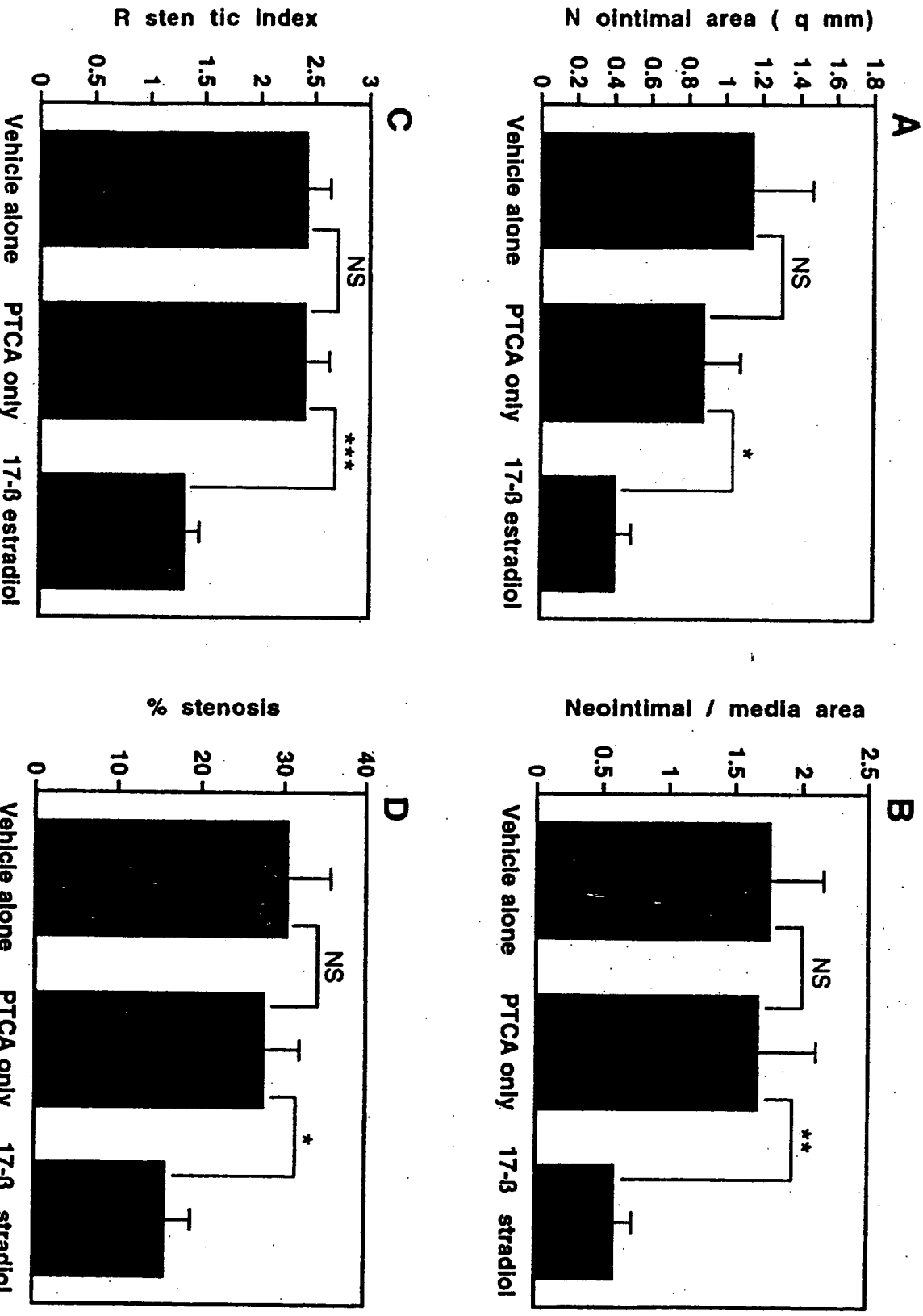


FIGURE 1C


A
N ointimal area (q mm)
0
0.2
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0.8
1
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Vehicle alone
PTCA only
17-ß estradiol
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*
B
Neointimal / media area
0
0.5
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1.5
2
2.5
Vehicle alone
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17-ß stradiol
NS
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C
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0.5
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3
Vehicle alone
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17-ß estradiol
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D
% stenosis
0
10
20
30
40
Vehicle alone
PTCA only
17-ß stradiol
NS
*

FIGURE 2

TITLE OF THE INVENTION

Local Delivery of 17 – beta Estradiol Decreases Neointimal Hyperplasia Following Coronary Angioplasty in Porcine Model

FIELD OF THE INVENTION

The present invention relates to the local use of estradiol for preventing restenosis. More specifically, the present invention is concerned with the local use of estradiol for decreasing neointimal hyperplasia that occurs during restenosis.

BACKGROUND OF THE INVENTION

Restenosis is currently the major limitation of percutaneous transluminal coronary angioplasty (PTCA), and is seen in up to 30-40 % of patients.[1]The most important mechanisms contributing to restenosis are neointimal proliferation, vascular remodelling, and elastic recoil.[2] Elastic recoil and vascular remodelling can be reduced to a large extent by stenting.[3] Although radiation therapy has been reported to show beneficial effeets,[4,5] no effective therapy exists yet for neointimal proliferation. Vascular smooth muscle cell (SMC) migration and proliferation have been documented to occur as early as 36 hours following arterial injury.[6] In cell culture assays, 17 - beta estradiol inhibited migration and proliferation of rat vascular SMC.[7,8] Similar effects have also been shown with human vascular SMC from saphenous vein.[9] Prolonged systemic administration of estrogen has been shown to inhibit intimal hyperplasia in animal

studies.[10,11] In the present experiment, we tested the hypothesis that local administration of 17 - beta estradiol during PTCA could effectively inhibit neointimal proliferation.

SUMMARY OF THE INVENTION

An object of the present invention is therefore to provide an efficient method by which 17-β estradiol is used locally during PTCA to prevent restenosis. Compositions for executing this method are also a further object of this invention.

Other objects, advantages and features of the present invention will become more apparent upon reading of the following non restrictive description of preferred embodiments thereof, given by way of example only with reference to the accompanying drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

In the appended drawings:

Figure 1 Representative light micrographs (x 40 magnification) of arterial segments from the same animal, stained with Verhoeff's stain. 17 - beta estradiol (a) treated segment shows markedly less neointimal hyperplasia compared to PTCA only (b), or vehicle alone (c) groups. The extent of injury is similar in all 3 segments.;

Figur 2 Comparison of (A) neointimal area, (B) neointimal/media area, (C) restenotic index, and (D) % stenosis between PTCA alon vs vehicle

only, and PTCA only vs 17 -beta estradiol groups; * $p < 0.05$, ** $p < 0,01$ *** $p < 0.002$. Values are expressed as mean ± SEM.

DESCRIPTION OF THE PREFERRED EMBODIMENT

Methods

Animal preparation

Eighteen juvenile farm pigs (9 female, and 9 castrated male) weighing 20 - 25 kg were studied. The study was approved by, and conducted in accordance with, the guidelines of the Animal Care and Ethical Research Committee of the Montreal Heart Institute. Before the procedure, animals were given 650 mg of acetylsalicylic acid and 30 mg of nifedipine orally, premedicated with intramuscular injection of 6 mg/kg of a mixture of tiletamine hydrochloride and zolazepam hydrochloride, and given 0.05 mg of atropine. The invasive procedure was performed under general anesthesia with a mixture of isoflurane (1 to 1.5 %) and oxygen enriched air. The right femoral artery was cannulated percutaneously, and an 8 Fr arterial sheath was introduced. After arterial access had been obtained, 100 mg of lidocaine and 250 U/kg of heparin were administered intra-arterially via the sheath. Activated coagulation time was maintained at > 300 seconds throughout the procedure.

Angioplasty and Local Delivery

Standard PTCA equipment was used. An 8 Fr right Amplatz guiding catheter and right Judkins guiding catheter were used for cannulation of

the left and right coronary arteries, respectively. PTCA was performed with a balloon size chosen to correspond to a balloon/artery ratio of 1.1-1.3. Three 30-second inflations at 10 atm pressure were performed with a 30-second interval between each inflation. Inflations were performed adjacent to major side branches to facilitate identification during harvesting, taking precaution not to include any side branch in the intended PTCA site. The left anterior descending, left circumflex, and right coronary arteries of each animal were subjected to PTCA. After PTCA, each coronary artery of an animal was randomized to receive either 600 µg of 17 - beta estradiol locally, or vehicle alone locally, or PTCA only. The chemicals 17 - beta estradiol and its vehicle 2-hydroxypropyl-beta-cyclodextrin (HPCD) were purchased from Sigma Chemical Co. The InfusaSleeve catheter (Local Med, Inc.) was used for local delivery. [12] Five ml of the designated substance was delivered at a driving pressure of 10 atm and support balloon pressure of 6 atm.

Of the 18 animals, 2 died a few days after PTCA, and were excluded; thus, 16 animals were analyzed. Twelve animals were euthanised at 28 days, and 4 at 7 days. After premedication and anesthesia, the right internal jugular vein and common carotid artery were cannulated. Following cross-clamping of the descending thoracic aorta exposed via a left lateral thoracotomy, exsanguination was performed, with simultaneous administration of 1 l of 0.9 % NaCl solution. The heart was perfusion-fixed in vivo with 2 1 of 10 % buffered formalin at 200 mm Hg pressure, removed from the animal, and placed in 10 % buffered formalin solution. Coronary arteries were then dissected free from surrounding tissues. The sit of PTCA was identified in relation to adjacent side branches, which

served as landmarks. The injured segment was harvested with a 1 cm normal segment proximal and distal to the injured site. Serial sections 3 to 5 mm long were made from the harvested segment, with a minimum of at least 3 sections (maximum 5) from each PTCA site. Sections were stored in buffered 10 % formalin and subjected to dehydration with increasing concentrations of alcohol, followed by treatment with xylene and paraffin. Each section was then cut to slices of 6 µm thickness with a microtome (Olympus cut 4060 E), and stained with Verhoeff's stain for morphometric analysis.

Morphometric analysis

Measurements were made with a video microscope (Leitz Diaplan, equipped with a Sony DXC 970 MD color video camera) linked to a 486 personal computer and customized software. A minimum of 3 sections for each injured segment were analyzed and results averaged. Analyses were made by a single observer unaware of the treatment group to which each segment had bee allocated. Randomly selected sections were viewed by a second observer (also blinded to protocol) independently; inter-observer variability was < 5 %. The areas of external elastic lamina (EEL), internal elastic lamina (IEL), and lumen were measured by digital planimetry; neointimal (I) area (IEL - lumen area) and media (M) area (EEL - IEL area) were obtained. The % neointima was defined as the % of total vessel area occupied by neointima (% neointima = [I/EEL] x 100). Morphologic % stenosis was calculated as 100 (1 – lumen/IEL area).[13] The restenotic index was defined as [I/(I + M)]/(F/IEL circumference), wher F is the

fracture length of internal elastic lamina. [14] Histologic injury score was determined as previously defined.[15]

Immunohistochemistry

Following slicing with a microtome and blocking of non-specific antibodies, the sections were treated with mouse anti - proliferating cell nuclear antigen (PCNA) antibodies and diluted biotynilated goat anti - mouse antibodies. They were then incubated with avidin -biotin (Elite ABC Kit, Vector Laboratories), and developed with 3, 3'- diaminobenzidine (Vector Laboratories). They were finally counter-stained with hematoxylin. Porcine liver cells were used as a positive control. For each section, a 6 µm slice counter-stained with hematoxylin without treatment with the primary antibody (mouse anti - PCNA) served as a negative control.

The proliferative response to injury was studied by immunohistochemical analysis of samples from animals euthanised at 7 days. The % proliferating SMC was obtained by dividing the number of PCNA - positive SMC by the total number of SMC in each field; separate measurements were made for neointimal and media layers. The proliferating cells were identified as SMC by positive staining of parallel sections with a-smooth muscle actin antibody. To standardize comparison among treatment groups, measurements were obtained at 4 fixed locations separated by 90° sites for each section, and the results averaged. For each segment, two sections demonstrating maximal neointimal response were analyzed, and the results averaged.

Statistical Analysis

Values are expressed as mean t standard deviation, except as otherwise indicated. Kruskal - Wallis analysis was used for comparison of data among the 3 groups; subsequently, 17 - beta estradiol and vehicle alone groups were separately compared with the PTCA only group using the Mann - Whitney rank sum test. Chi - square analysis was used for comparison of proportions. The Mann - Whitney rank sum test was also used for comparison of data between male and female animals within the 17 - beta estradiol treated group. Values were considered statistically significant if $p < 0.05$.

Results

Following PTCA and local delivery, animals were allowed to recover, and gained weight steadily. Two animals died 48 and 72 hours after procedure respectively, and were not included; thus 16 animals were studied. Autopsy of the 2 animals revealed occlusive thrombus at the site of PTCA (in the 17 - beta estradiol treated vessel in one pig, and in the vessel treated with PTCA only in the other pig).

Injured segments

Balloon/artery ratio and artery diameter were not significantly different among the 3 treatment groups (Table 1). Segments with intact IEL in which discernible injury was absent wer xcluded from analysis (2 from PTCA only group, and 1 from vehicle alone group). Two segments wer

lost during harvesting and processing (1 of vehicle alone, and 1 of PTCA only group).

Morphometric analysis

Of the 12 animals that underwent morphometric analysis at 28 days, arterial segments treated with local delivery of 17 - beta estradiol showed significantly less neointimal hyperplasia (Figure 1). This beneficial effect was noted in all parameters of neointimal response to injury that were analyzed (Table 1). Of note, the extent of morphologic injury was similar among the 3 groups, suggesting that the use of the InfusaSleeve catheter was not associated with an enhanced risk of injury.

It was important to exclude an inhibitory effect on intimal proliferation due to the vehicle, and, to confirm that the effect noted was in response to treatment with 17 - beta estradiol. Analyses comparing segments treated with vehicle alone and PTCA only showed a similar response in terms of the extent of neointimal proliferation. On the other hand, significantly less intimal hyperplasia was observed in 17 - beta estradiol treated segments as compared to segments treated with PTCA only (Figure 2). Compared to PTCA only, or vehicle alone, 17 - beta estradiol decreased neointima formation by 54.6 % and 64.9 % respectively.

To exclude the possibility of influence of sex on response to estrogen, the 7 segments obtained from male pigs treated with 17 - beta estradiol, and 5 segments obtained from female pigs treated with 17 - beta estradiol

were analyzed. No statistically significant differences were evident (Table 2).

Immunohistochemistry

The number of PCNA - positive SMC was low overall; sacrifice at an earlier time might have yielded a higher number. However, a statistically significant decrease in the proliferative response was seen in animals treated with 17 - beta estradiol. Among the different groups, the % of PCNA - positive SMC in the neointima were 0.43 ± 0.52 % in 17 - beta estradiol, 4.26 ± 2.33 % in PTCA only, and 4.27 ± 2.73 % in vehicle alone groups respectively ($p < 0.05$ for 17 - beta estradiol vs other 2 groups). There were no statistically significant differences in % PCNA - positive SMC in the media among the 3 groups: 0.4 ± 0.3 %, 1.38 ± 1.74 %, and 1.24 ± 1.57 % for 17 - beta estradiol, PTCA only, and vehicle alone groups respectively (p = NS).

Vascular remodeling

To determine the effect on vascular remodeling of the agents used, the EEL area of the injured segment and of the normal vessel proximal to site of PTCA were obtained, and their ratio calculated. [13] No significant difference among the groups was noted: 1.01 ± 0.16, 1.16 ± 0.28, 1.31 ± 0.37 respectively for 17 - beta estradiol, PTCA only, and vehicle alone groups respectively (p = NS).

Discussion

The present study demonstrates, for the first time, that locally delivered 17 - beta estradiol decreases neointimal proliferation following PTCA in pigs. The study also shows that the InfusaSleeve catheter can be used to deliver effectively 17 - beta estradiol intramurally in coronary arteries.

Several previous experiments in animals have demonstrated that estrogen administered subcutaneously for up to 3 weeks inhibited the myointimal response to arterial injury. [10,11] Recently, short-term subcutaneous estrogen therapy (6 to 17 days) was also shown to be effective in reducing the injury response in rat carotid artery. [16] Estrogen administered intramuscularly for at least 3 weeks has also demonstrated the potential to inhibit vascular smooth muscle cell proliferation and neointimal hyperplasia in rabbits. [17] However, the efficacy of local delivery of 17 - beta estradiol to inhibit intimal hyperplasia has not been previously studied.

The biologic effects of estrogen, like other steroid hormones, involve intracellular receptors. The first estrogen receptor (ER) to be discovered was ERα,[18,19] which was thought to mediate the beneficial effects of estrogen following vascular injury. ERα was also present in coronary arteries obtained from autopsy specimens in both pre- and post-menopausal women,[20] and in cell cultures of human saphenous vein and internal mammary artery specimens.[21] Recently, a second estrogen receptor, ERβ, has been identified in animals and humans.[22,23] The role of ERβ in response to vascular injury was subsequently demonstrated in experiments with ERα deficient mice. [24] Normal and ERα deficient mice treated with estrogen, when subjected to arterial injury, showed the same

extent of inhibition of neointimal proliferation compared to control mice; thereby demonstrating that inhibition of vascular injury response by estrogen is independent of ERα. Although the present experiment was not designed to study the mechanism of action of 17 - beta estradiol, evidence exists for multiple potential mechanisms by which 17 - beta estradiol can inhibit the vascular response to injury. Of importance may be the effect of 17 - beta estradiol on nitric oxide (NO) synthesis. In cell culture studies with human and bovine endothelial cells, treatment with 17 - beta estradiol stimulated NO synthase and increased NO production.[25,26] Postmenopausal women treated with transdermal 17 - beta estradiol showed enhanced in vivo NO synthesis.[27] NO has demonstrated inhibitory effects on both migration [28] and proliferation [29] of vascular SMC, and decreased neointima formation after PTCA.[13] Preliminary reports have shown that therapy with 17 - beta estradiol decreases intercellular and vascular cell adhesion molecule expression by human coronary SMC.[30] Cellular adhesion molecules are expressed by SMC following arterial injury[31] and their suppression with the use of monoclonal antibodies inhibited intimal hyperplasia after arterial injury in rats.[32] The regulatory effect of 17 - beta estradiol on vascular endothelial growth factor expression may also be partly responsible.[33-35] Perhaps the most important mechanism may be a direct inhibitory effect of 17 - beta estradiol on vascular SMC proliferation.[36] The binding of 17 - beta estradiol to its intracellular receptor activates DNA containing "estrogen responsive elements", leading to altered gene expression. 17 - beta estradiol also reduces platelet derived growth factor - induced migration and proliferation of vascular SMC.[9]

The beneficial effects of 17 - beta estradiol, the predominant circulating estrogen in premenopausal women, on vascular injury response may not be replicated by other kinds of estrogens; for example, conjugated equine estrogen was found to have no effect on neointimal proliferation in non-human primate models.[37] Simultaneous administration of progesterone may attenuate the vascular injury response to 17 - beta estradiol.[38] A sexually dimorphic response to estrogen in intact rats has been reported following arterial injury, with male rats deriving no benefit with estrogen therapy .[39] This sexually dimorphic effect was, however, not observed in another experiment with gonadeetomized rats.[11] I In the present study, too, no significant difference in neointimal proliferative response to 17 - beta estradiol was noted between the sexes. Increased expression of ERβ mRNA (ERβ is directly associated with inhibition of vascular SMC proliferation) following arterial injury has been demonstrated in intact male rats;[40] of additional interest in the study is that no increase in ERα was seen following arterial injury.

17 - beta estradiol is a lipophilic compound with poor solubility in aqueous solutions, thereby needing a vehicle for parenteral administration. HPCD is a starch derivative that has been successfully tested as an effective excipient for protein drugs. [41] The pharmacokinetics of HPCD are similar to that of inulin, and the toxic dose (nephrotoxicity) has been estimated to be 200 mg/kg in rats.[42] The dose of HPCD used to dissolve 17 - beta estradiol in the present study was 0.63 mg/kg, far below the toxic dose. Furthermore, HPCD has been used for administration of ophthalmic preparations and intravenous anaesthetic agents in humans.[43,44] HPCD complexed to 17 - beta estradiol has been used to enhance bioavailability

of orally, or, sublingually administered 17 - beta estradiol with no untoward effects in humans.[45]

Retrospective studies in humans have shown no benefit of hormonal replacement therapy on angiographic restenosis following PTCA;[46] although one study did show a beneficial effect after directional atherectomy.[47] However, it should be noted that conjugated estrogen (and not 17 - beta estradiol) was the predominant form of estrogen used in many of these patients, and, no information about concommittent use of progesterone is available.

In conclusion, we have shown that, a single dose of 17 - beta estradiol delivered locally during PTCA has the potential to inhibit neointimal proliferation effectively. The delivery of 17 - beta estradiol can be performed easily with the InfusaSleeve catheter, without risk of additional injury. With this approach, it may be possible to avoid potential undesirable effects of long term systemic administration of estrogen. ERβ has been identified in humans, and inhibition of proliferation of human vascular SMC by 17 - beta estradiol has been demonstrated in cell culture assays. The local administration of 17 - beta estradiol is therefore a promising new approach, which might be useful in preventing the proliferative response after PTCA in humans. Its usefulness in preventing restenosis after PTCA merits further investigation.

References

1. Dangas G, Fuster V. Management of restenosis after coronary intervention. Am Heart J 1996 ;132 :428-36.

2. Post MJ, Borst C, Kuntz RE. The relative importance of arterial remodelling compared with intimal hyperplasia in lumen narrowing after balloon angioplasty. Circulation 1994; 89: 2816-21.

3. Currier JW, Faxon DP. Restenosis after percutaneous transluminal coronary angioplasty: Have we been aiming at the wrong target? J Am Coll Cardiol 1995; 25: 516-20.

4. Teirstein PS, Massullo V, Jani S, Popma JJ, Mintz GS, Russo RJ, Schatz RA, Guarneri EM, Steuterman S, Morris NB, Leon MB, Tripuraneni P. Catheter-based radiotherapy to inhibit restenosis after coronary stenting. N Engl J Med 1997; 336: 1697-703.

5. King SBIII, Williams DO, Chougule P, Klein JL, Waksman R, Hilstead R, Macdonald J, Anderberg K, Crocker IR. Endovascular beta-radiation to reduce restenosis after coronary balloon angioplasty: results of the Beta Energy Restenosis Trial (BERT). Circulation 1998; 97: 2025-30.

6. Clowes AW, Reidy MA, Clowes MM. Kinetics of cellular proliferation after arterial injury: smooth muscle cell growth in the absence of endothelium. Lab Invest 1983; 49: 327-3 3 .

7. Akishita M, Ouchi Y, Miyoshi H, Kozaki K, Inoue S, Ishikawa M, Eto M, Toba K, Orimo H. Estrogen inhibits cuff- induced intimal thickening of rat femoral artery: effects on migration and proliferation of vascular smooth muscle cells. Atherosclerosis 1997; 130: 1-10.

8. Kolodgie FD, Jacob A, Wilson PS, Carlson GC, Farb A, Verma A, Virmani R. Estradiol attenuates directed migration of vascular smooth muscle cells *in vitro*. Am J Pathol 1996; 148: 969-76.

9. Dai - Do D, Espinosa E, Liu G, Rabelink TJ, Julmy F, Yang Z, Mahler F, Luscher TF. 17 - beta estradiol inhibits proliferation and migration of human vascular smooth muscle cells: similar effects in cells from postmenopausal females and in males. Cardiovascular Research 1996; 32: 980-5.

10. Sullivan Jr TR, Karas RH, Aronovitz M, Faller GT, Ziar JP, Smith JJ, O'Donnell Jr TF, Mendelsohn ME. Estrogen inhibits the response - to - injury in a mouse carotid artery model. J Clin Invest 1995; 96: 2482-8.

11. Chen SJ, Li H, Durand J, Oparil S, Chen YF. Estrogen reduces myointimal proliferation after balloon injury of rat carotid artery. Circulation 1996; 93: 577-84.

12. Moura A, Lam JYT, Hebert D, Kermode JR, Grant GW, Robitaille D, Klein EJ, Yock PG, Simpson JB, Kaplan AV. Intramural delivery of agent via a novel drug - delivery sleeve: histological and functional evaluation. Circulation 1995; 92: 2299-2305.

13. Varenne O, Pislaru S, Gillijns H, Pelt NV, Gerard RD, Zoldhelyi P, Van de Werf F, Collen D, Janssens S. Local adenovirus - mediated transfer of human endothelial nitric oxide synthase reduces luminal narrowing after coronary angioplasty in pigs. Circulation 1998; 98: 916-26.

14. Bonan R, Paiement P, Scortichini D, Cloutier MJ, Leung TK. Coronary restenosis: evaluation of a restenosis injury index in a swine model. Am Heart J 1993; 126: 1334-40.

15. Karas SP, Gravanis MB, Santoian EC, Robinson KA, Anderberg KA, King III SB. Coronary intimal proliferation after balloon injury and stenting in swine: an animal model of restenosis. J Am Coll Cardiol 1992; 20: 467-74.

16. Mori T, Durand J, Chen YF, Thompson JA, Oparil S. Short term estrogen treatment prior to and following balloon injury of rat carotid artery effectively blunts the vascular injury response. J Am Coll Cardiol 1999; 33 (2 suppl A): 259A (abstract).

17. Foegh ML, Asotra S, Howell MH, Ramwell PW. Estradiol inhibition of arterial neointimal hyperplasia after balloon injury. J Vasc Surg 1994; 19(4): 722-6.

18. Colburn P, Buonassis V. Estrogen - binding sites in endothelial cell cultures. Science 1978; 201: 817-9.

19. Venkov CD, Rankin AB, Vaughan DE. Identification of authentic estrogen receptor in cultured endothelial cells: a potential mechanism for steroid hormone regulation of endothelial function. Circulation 1996; 94: 727-33.

20. Losordo DW, Kearney M, Kim EA, Jekanowski J, Isner JM. Variable expression of the estrogen receptor in normal and atherosclerotic coronary arteries of premenopausal women. Circulation 1994; 89: 1501-10.

21. Karas RH, Patterson BL, Mendelsohn ME. Human vascular smooth muscle cells contain functional estrogen receptor. Circulation 1994; 89: 1943-50.

22. Kuiper CiGMJ, Enmark E, Pelto - Huikko M, Nilsson S, Gustafsson JA. Cloning of a novel estrogen receptor expressed in rat prostrate and ovary. Proc Natl Acad Sci USA 1996; 93: 5925-5930.

23. Mosselman S, Polman J, Dijkema R. ER/3: identification and characterization of a novel human estrogen receptor. FEBS Left 1996; 392: 49-53.

24. Iafrati MD, Karas RH, Aronovitz M, Kim S, Sullivan Jr TR, Lubahn DB, O'Donnell Jr TF, Korach KS, Mendelsohn ME. Estrogen inhibits the vascular injury response in estrogen receptor a-deficient mice.

25. Hishikawa K, Nakaki T, Marumo T, Suzuki H, Kato R, Saruta T. Up - regulation of nitric oxide synthase by estradiol in human aortic endothelial cells. FEBS Lett 1995; 360: 291-3.

26. Hayashi T, Yamada K, Esaki T, Kuzuya M, Satake S, Ishikawa T, Hidaka H, Iguchi A. Estrogen increases endothelial nitric oxide by a receptor - mediated system. Biochem Biophys Res Commun 1995; 214(3): 847-55.

27. Rosselli M, Imthurn B, Keller PJ, Jackson EK, Dubey RK. Circulating nitric oxide (nitrite/nitrate) levels in postmenopausal women substituted with 170-estradiol and norethisterone acetate: a two-year follow-up study. Hypertension 1995; 25(part 2): 848-53.

28. Sarkar R, Meinberg EG, Stanley JC, Gordon D, Webb RC. Nitric oxide reversibly inhibits the migration of cultured vascular smooth muscle cells. Circ Res 1996; 78: 225-230.

29. Cornwell TL, Arnold E, Boerth NJ, Lincoln TM. Inhibition of smooth muscle cell growth by nitric oxide and activation of cAMP-dependent protein kinase by cGMP. Am J Physiol 1994; 267: C1405-13.

30. Speir E, Yu ZX, Ferrans VJ, Cannon III RO. Estrogen inhibits transcription factor and cell adhesion molecule activation in cytokine-stimulated human coronary smooth muscle cell via antioxidant effects. Circulation 1998; suppl I: I-220 (abstract).

31. Tanaka H, Sukhova GK, Swanson SJ, Clinton SK, Ganz P, Cybulsky MI, Libby P. Sustained activation of vascular cells and leucocytes in the rabbit aorta after balloon injury. Circulation 1993; 88: 1788-1803.

32. Yasukawa H, Imaizumi T, Matsuoka H, Nakashima A, Morimatsu M. Inhibition of intimal hyperplasia after balloon injury by antibodies to intercellular adhesion molecule-1 and lymphocyte function - associated antigen-1. Circulation 1997; 95: 1515-22.

33. Hyder SM, Stancel GM, Chiappetta C, Murthy L, Boettger-Tong HL, Makela S. Uterine expression of vascular endothelial growth factor is increased by estradiol and tamoxifen. Cancer Res 1996; 56(17): 3954-60.

34. McLaren J, Prentice A, Charnock-Jones DS, Millican SA, Muller KH, Sharkey AM, Smith SK. Vascular endothelial growth factor is produced by peritoneal fluid macrophages in endometriosis and is regulated by ovarian steroids. J Clin Invest 1996; 98: 482-9.

35. Asahara T, Bauters C, Pastore C, Kearney M, Rossow S, Bunting S, Ferrara N, Symes JF, Isner JM. Local delivery of vascular endothelial growth factor accelerates reendothelialization and attenuates intimal hyperplasia in balloon-injured rat carotid artery. Circulation 1995; 91: 2793-2801.

36. Mendelsohn ME, Karas RH. Estrogen and the blood vessel wall. Current Opinion in Cardiology 1994; 9: 619-26.

37. Geary RL, Adams MR, Benjamin ME, Williams JK. Conjugated equine estrogens inhibit progression of atherosclerosis but have no effect an intimal hyperplasia or arterial remodelling induced by balloon catheter injury in monkeys. J Am Coll Cardiol 1998; 31: 1158-64.

38. Levine RL, Chen SJ, Durand J, Chen YF, Oparil S. Medroxyprogesterone attenuates estrogen-mediated inhibition of neointima formation after balloon injury of the rat carotid artery. Circulation 1996; 94: 2221-7.

39. Oparil S, Levine RL, Chen SJ, Durand J, Chen YF. Sexually dimorphic response of the balloon-injured rat carotid artery to hormone treatment. Circulation 1997; 95: 1301-7.

40. Lindner V, Kim SK, Karas RH, Kuiper GGJM, Gustafsson JA, Mendelsohn ME. Increased expression of estrogen receptor-f3 mRNA in male blood vessels after vascular injury. Circ Res 1998; 83: 224-9.

41. Brewster ME, Hora MS, Simpkins JW, Bodor N. Use of 2-hydroxypropyl-betacyclodextrin as a solubilizing and stabilizing excipient for protein drugs. Pharm Res 1991; 8(6): 792-5.

42. Frijlink HW, Visser J, Hefting NR, Oosting R, Meijer DKF, Lerk CF. The pharmacokinetics of beta-cyclodextrin and 2-hydroxypropyl-beta-cyclodextrin in the rat. Pharm Res 1990; 7(12): 1248-52.

43. Kristinsson JK, Fridriksdottir H, Thorisdottir S, Sigurdardottir AM, Stefansson E, Loftsson T. Dexamethasone-cyclodextrin-polymer co-complexes in aqueous eye drops: aqueous humor pharmacokinetics in humans. Invest Ophthalmol Vis Sci 1996; 37: 1199-1203.

44. Doenicke A, Roizen MF, Nebauer AE, Kugler A, Hoernecke R, Beger-Hintzen H. A comparison of two formulations for etomidate, 2-hydroxypropyl-beta-cyclodextrin (HPCD) and propylene glycol. Anesth Analg 1994; 79: 933-9.

45. Hoon TJ, Dawood Y, Khan-Dawood FS, Ramos J, Batenhorst RL. Bioequivalence of a 17 - beta estradiol hydroxypropyl-beta-cyclodextrin complex in postmenopausal women. J Clin Pharmacol 1993; 33: 1116-21.

46. O'Keefe JH, Kim SC, Hall RR, Cochran VC, Lawhorn SL, McCallister BD. Estrogen replacement therapy after coronary angioplasty in women. J Am Coll Cardiol 1997; 29: 1-5.

47. O'Brien JE, Peterson ED, Keeler GP, Berdan LG, Ohman EM, Faxon DP, Jacobs AK, Topol EJ, CaliffRM. Relation between estrogen replacement therapy and restenosis after percutaneous coronary interventions. J Am Coll Cardiol 1996; 28: 1111-8.

Table 1. Morphometric Analysis

Characteristics	17 - beta estradiol	PTCA only	Vehicle alone	p value*
Segments analyzed	12	9	10	NS
Artery size (mm)	2.86 ± 0.35	2.94 ± 0.24	2.94 ± 0.41	NS
Balloon/Artery ratio	1.22 ± 0.09	1.2 ± 0.06	1.17 ± 0.11	NS
EEL_{ref}/EEL_{inj} †	1.01 ± 0.16	1.31 ± 0.37	1.16 ± 0.28	NS
Neointimal area (mm^2)	0.4 ± 0.3	0.88 ± 0.61	1.14 ± 1.03	< 0.05
% neointima	12.16 ± 8.89	23.02 ± 11.91	25.46 ± 14.96	< 0.025
Neointima/Media area	0.59 ± 0.48	1.67 ± 1.29	1.75 ± 1.29	< 0.01
% stenosis	15.67 ± 11.13	27.51 ± 13.17	30.34 ± 17.05	< 0.025
Restenotic index	1.3 ± 0.5	2.4 ± 0.68	2.42 ± 0.71	< 0.005
Injury score	1.64 ± 0.34	1.7 ± 0.43	1.77 ± 0.47	NS

* 17 - beta estradiol vs other 2 groups; †EEL_{ref} = proximal reference segment external elastic lamina area, EEL_{inj} = injured segment external elastic lamina area (averaged).

Table 2. Response to 17 - beta estradiol According to Sex of the Animal

Characteristics	Male	Female	p value
Restenotic index	1.2 ± 0.59	1.37 ± 0.45	> 0.1
Neointimal area (mm^2)	0.51 ± 0.34	0.25 ± 0.15	> 0.1
Neointima/Media area	0.78 ± 0.55	0.32 ± 0.16	> 0.1
% neointima	14.93 ± 10.68	8.29 ± 3.72	> 0.1
% stenosis	18.93 ± 13.39	11.09 ± 5.16	> 0. I

Although the present invention has been described hereinabove by way of preferred embodiments thereof, it can be modified, without departing from the spirit and nature of the subject invention as defined in the appended claims.

WHAT IS CLAIMED IS:

1. The use of 17-β estradiol in the making of a medication to prevent restenosis, for *in situ* administration at a site susceptible to restenosis.

2. A composition for preventing restenosis comprising an anti-vascular smooth cell proliferative amount of 17-β estradiol in a pharmaceutically acceptable carrier.

3. A device comprising 17-β estradiol for *in situ* delivery to a vascular site susceptible to restenosis.

ABSTRACT OF THE DISCLOSURE

The cardioprotective effects of estrogen are well recognized. In *in vitro* experiments, and upon systemic administration, 17 - beta estradiol has shown to inhibit vascular smooth muscle cell proliferation and intimal hyperplasia. We hypothesized that locally delivered 17 - beta estradiol could inhibit neointimal proliferation following balloon angioplasty in porcine coronary arteries. Immunohistochemical, and morphometric analyses revealed that, arterial segments treated with local delivery of 17 - beta estradiol showed significantly less smooth muscle cell proliferation and neointima formation. Compared to PTCA only, or vehicle alone, 17 - beta estradiol decreased neointima formation by 54.6 % and 64.9 % respectively.